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TITLE: The Therapeutic Effect of the Antitumor Drug 11beta and Related Molecules on Polycystic Kidney Disease

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14. ABSTRACT
This project aims to develop synthetic multifunctional compounds as therapeutics for polycystic kidney disease (PKD). In collaboration with Somlo group at Yale University, we have already shown that two parent compounds, 11β-dichloro and 11β-dipropyl, are effective at preventing and delaying cystic growth in two different mouse models of PKD. One arm of the project focuses on the synthesis of new molecules from the 11β family, which will inform, through a structure-activity study, the key molecular features required for activity and provide additional hints about the mechanism of action. A second arm of the project focuses on the development of a cell culture model that can be used to screen the new molecules for improved efficacy and selectivity; such molecules will be then validated in the established PKD mouse models and pave the way towards their preclinical and clinical development. During the last funding period, we continued the work on validating cell culture models that recapitulate the biological consequences of 11β compounds. The pig kidney cell lines derived from LLC-PK1 show good promise for toxicity assays, when coupled with the total cellular ATP measurements (Cell-Titer Glo Assay). We also investigated aspects of the mechanism of toxicity in these cell culture models. Additionally, we completed the synthesis of 5 new 11β analogs, and a 6th analog will be completed soon. The new compounds were tested for efficacy and selectivity in cell culture, providing new structure-activity information. Moreover, our collaborators at Yale University continued their work probing the mechanism of toxicity of 11β compounds in animals (induction of mitochondrial ROS) and testing the 11β-dipropyl compound in the adult mouse model of PKD; only preliminary data are available at this point.

15. SUBJECT TERMS
Polycystic kidney disease, ADPKD, PKD1, polycystin1, therapeutic, apoptosis, mitochondria, reactive oxygen species, unfolded protein response

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1. INTRODUCTION

This project aims to develop synthetic multifunctional compounds as therapeutics for polycystic kidney disease (PKD). In collaboration with Somlo group at Yale University, we have already shown that two parent compounds, 11β-dichloro and 11β-dipropyl, are effective at preventing and delaying cystic growth in two different mouse models of PKD. To guide the development of new compounds, the mechanism by which 11β compounds achieve their efficacy and selectivity against cystic cells is also investigated. One arm of the project focuses on the synthesis of new molecules from the 11β family, which will inform, through a structure-activity study, the key molecular features required for activity and provide additional hints about the mechanism of action. A second arm of the project focuses on the development of a cell culture model that can be used to screen the new molecules for improved efficacy and selectivity; such molecules will be then validated in the established PKD mouse models and pave the way towards their preclinical and clinical development.

During the last funding period, we continued the work on validating cell culture models that recapitulate the biological consequences of 11β compounds. The pig kidney cell lines derived from LLC-PK1 show good promise for toxicity assays, when coupled with the total cellular ATP measurements (Cell-Titer Glo Assay). We also investigated aspects of the mechanism of toxicity in these cell culture models. Additionally, we completed the synthesis of 5 new 11β analogs, and a 6^{th} analog will be completed soon. The new compounds were tested for efficacy and selectivity in cell culture, providing new structure-activity information. Moreover, our collaborators at Yale University continued their work probing the mechanism of toxicity of 11β compounds in animals (induction of mitochondrial ROS) and testing the 11β-dipropyl compound in the adult mouse model of PKD; only preliminary data are available at this point.

2. KEYWORDS

Polycystic kidney disease, cystic disease, ADPKD, PKD1, PKD2, therapeutic, polycystin1, apoptosis, mitochondria, reactive oxygen species, unfolded protein response, mouse model.

3. ACCOMPLISHMENTS

What were the major goals of the project?

Our major goals were:

- Synthesize and characterize 11β analogs with different linkers and/or alkyl substituents (90% completed)
- Using the cell culture model, evaluate the efficacy and selectivity of the 11β analogs. In a structure-activity study (90% completed)
- Using the cell culture model, investigate the role of apoptosis, mitochondrial metabolism and UPR (unfolded protein response) in the toxicity and anti-PKD effects of 11β compounds (50% completed).
- Test 11β-dipropyl in the adult onset PKD mouse model (50% completed).

What was accomplished under these goals?

a. Synthesis and characterization of 11β analogs. (Essigmann, MIT)

One central goal of this project was to synthesize a series of 11β analogs and perform a structure-activity study that will inform which chemical and structural features of the molecule are essential for activity. Additional goals of this effort were to obtain a better lead compound with perhaps a simpler chemical structure. To this end, we varied the structure of the linker (carbamate, secondary amine or both) and the structure of the aniline moiety. The 11β analogs synthesized are shown in Figure 1.

Although not originally proposed, we synthesized an additional analog (compound **5**), in which the dipropyl arms of the 11β-dipropyl are replaced with methyl groups. This analog constitutes the smallest variation from the 11β-dipropyl structure, and its synthesis was facilitated by readily-available starting materials.

The synthetic schemes used for the synthesis of compounds **1-5** are shown in Figures 2 and 3. Except for compound **6**, which is anticipated to be completed within 1-2 months, all compounds were synthesized, isolated and purified with yields >100 mg each (Table 1). All compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, MS and UV-Vis.

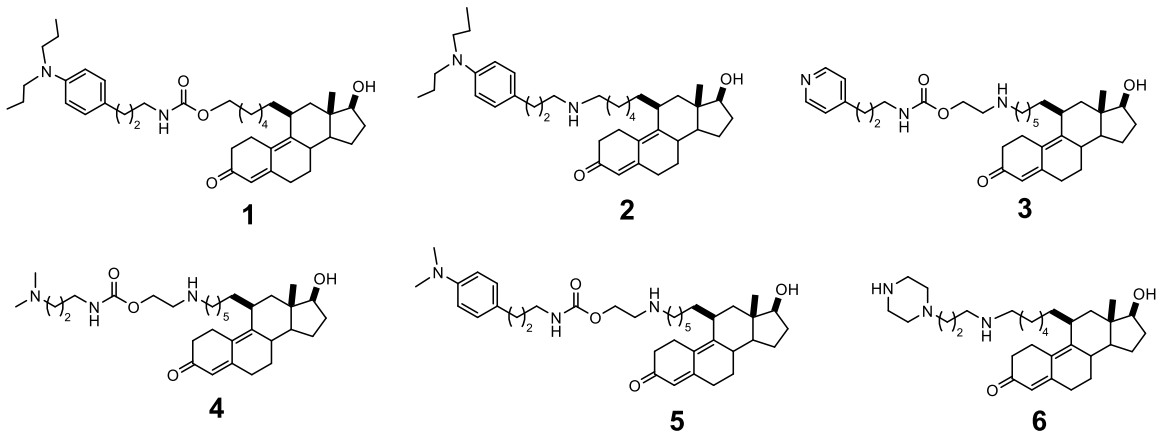

Figure 1. Chemical structures of the 11β-dipropyl analogs synthesized for structure-activity studies.

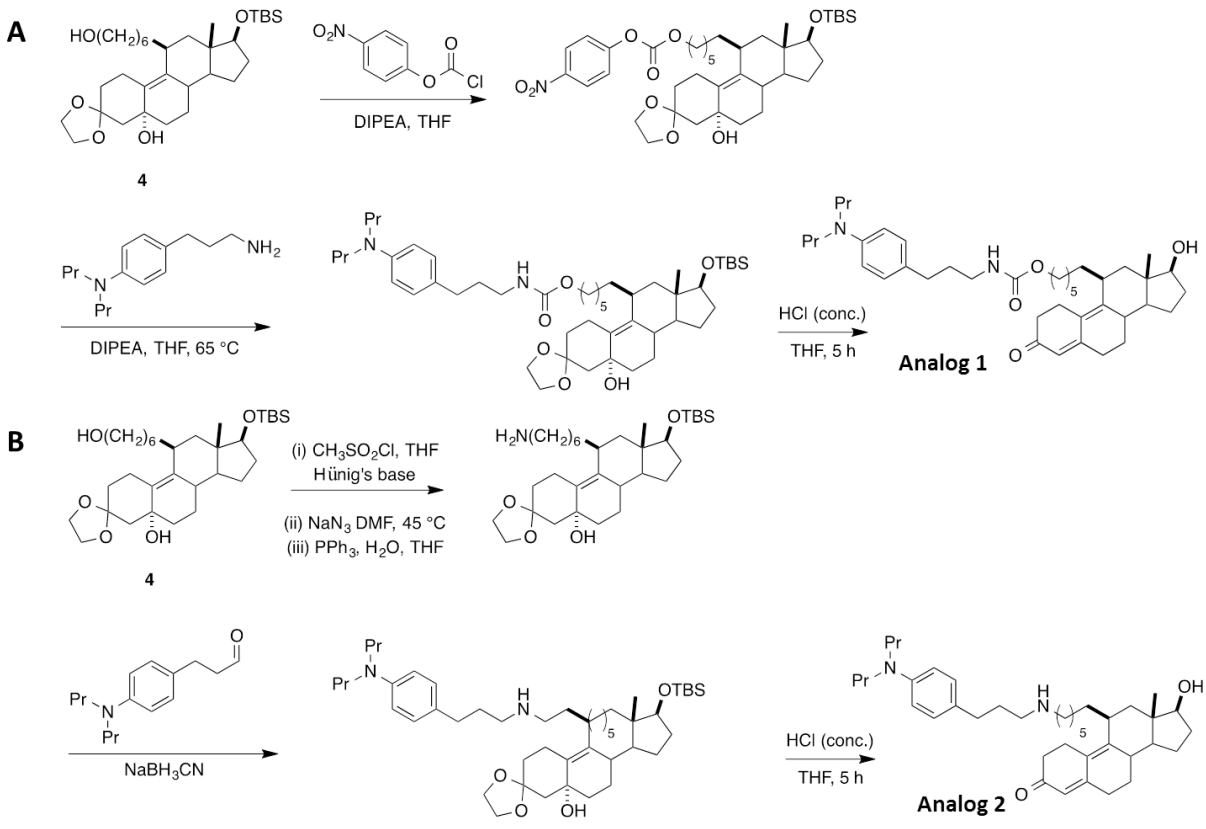

Figure 2. Chemical synthesis schemes for 11β-dipropyl analogs. A) The synthesis scheme for analog **1**, which features a linker with only the carbamate moiety. B) The synthesis scheme for analog **2**, which features a linker with only the secondary amine moiety.

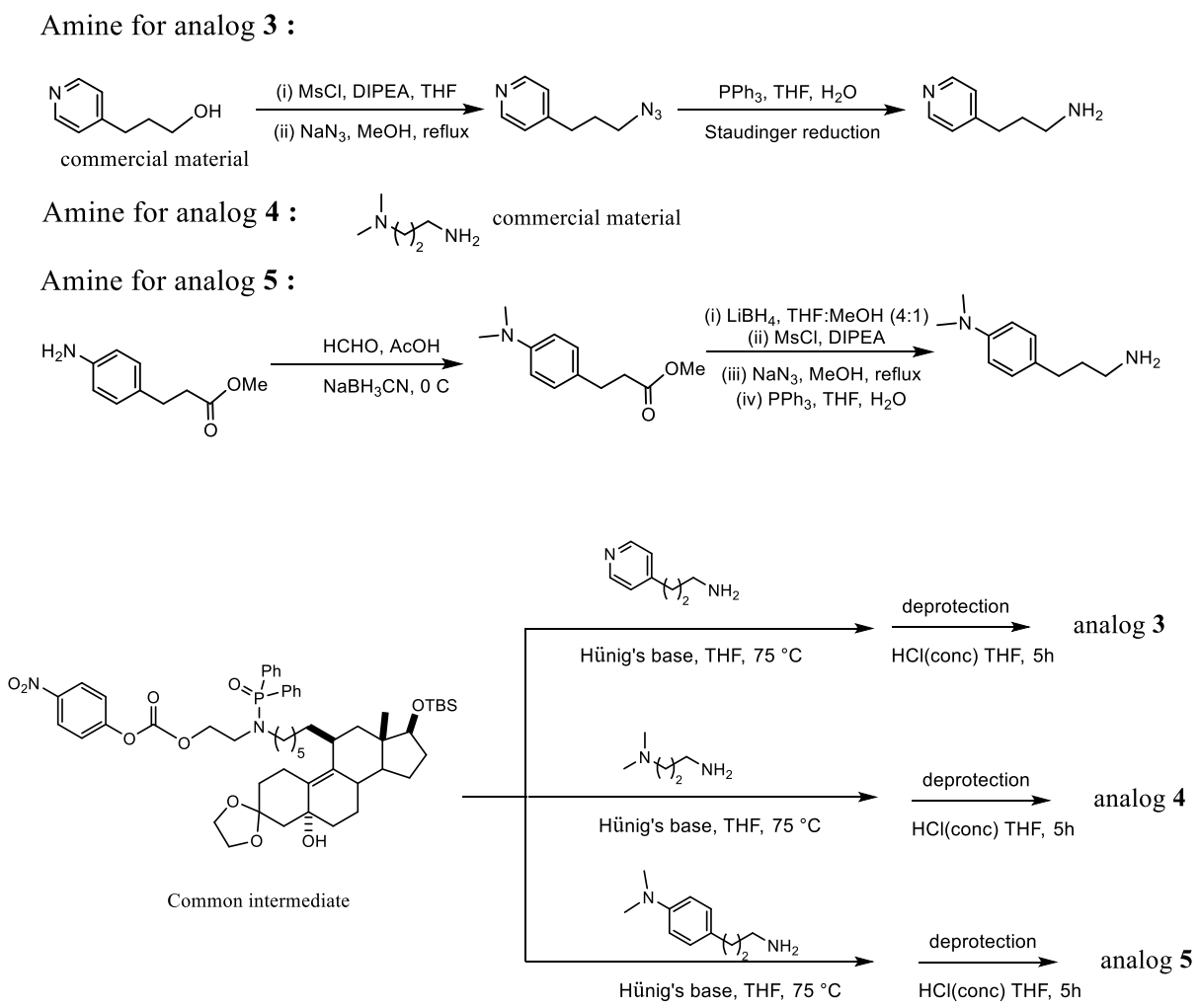

Figure 3. Chemical synthesis schemes for 11β-dipropyl analogs **3-5**.

b. Physico-chemical characterization of the 11β-dipropyl analogs. (Essigmann, MIT)

All the logP values have been estimated using the tools available at www.molinspiration.com, which estimate logP based on the equation of Horvath et al. (1, 2). The pKa values for all compounds were estimated using the Evans pKa tables available at www.evans.rc.fas.harvard.edu. The compounds were dissolved in pure ethanol and their UV-Vis spectra were recorded. All compounds showed the absorption peak at 305 nm, characteristic of the steroid moiety; however, the experimentally-determined extinction coefficient at 305 nm varied considerably across compounds (Table 1).

Table 1. Physico-chemical properties of 11β-dipropyl analogs. Structures are shown in Figure 1.

Compound	Amount synthesized (mg)	MW (g/mol)	logP	pKa	Extinction Coefficient at 305 nm (M^{-1} cm^{-1})
1	568	632.92	8.62	11	5752
2	107	588.91	8.36	10.5	4415
3	120	577.80	5.14	11	24390
4	127	543.78	4.41	11	32100
5	116	619.88	6.53	11	33030
6	*N/A*	497.76	3.84	10.5	*N/A*
11β-dipropyl	800	675.88	8.25	11	22500

c. Structure-activity study of 11β-analogs in the LLC-PK cell line model.

The commercially available kidney cell line LLC-PK1 (ATCC CL-101) was evaluated over the previous funding periods as a possible cell culture model for testing 11β compounds activity. LLC-PK1 is a porcine renal proximal tubule cell line from the Hampshire pig (3) that is routinely used to study nephrotoxicity. The advantages of this line over other cell lines are as follows: i) a stable kidney epithelium cell line; ii) the cells exhibit lateral growth inhibition, which allows formation of stable monolayers, without the need to inactivate a growth-driving oncogene (e.g. SV40); iii) easy to culture under standard conditions. The team at Yale then employed CRISPR-Cas9 technology to generate two isogenic cell lines derived from LLC-PK1 in which either PKD1 or PKD2 genes were deleted. The wild-type and the two mutant cell lines were then evaluated in cell culture by the Essigmann team at MIT.

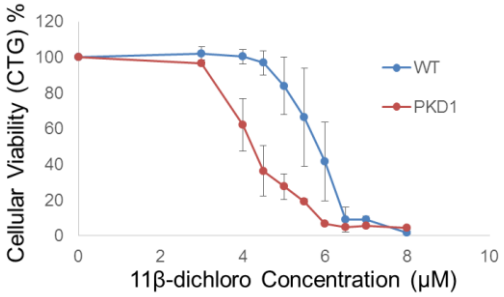

Figure 4. Dose responses to 11B-dichloro of LLC-PK cell lines (WT and PKD1$^{-/-}$).

Preliminary work with the LLC-PK cell lines showed that they could be a good cell culture model for testing sensitivity and specificity to 11β compounds. A dose response with 11β-dichloro showed that the PKD1 KO is more sensitive than the wild-type parent line (Figure 4). The cellular viability in this case was measured using the CellTiter-Glo reagent from Promega, which gives a luminescent signal proportional to the amount of ATP present in each well. For reasons that are currently under investigation, the CellTiter-Blue reagent we used for measuring the viability in other cell lines in previous studies (4) did not work with the LLC-PK cell lines.

Dose-response experiments were set up using the LLC-PK1 cell lines and the 11β analogs. The initial experiment used doses between 0-10 μM. Under these conditions, only compounds **5** and 11β-dipropyl showed substantial toxicity. Subsequently, the doses tested for compounds **1-4**

were increased (up to 300 μM) to determine their toxic range. Of these, compound **1** did not show any toxicity at concentrations as high as 300 μM. Based on the dose response curves, IC50 values (concentration at which cell viability is decreased by 50%) were estimated (Table 2).

Table 2. The toxicity (IC50 concentrations) of 11β analogs in the LLC-PK1 cell line model.

Compound	$IC50_{WT}$ (μM)	$IC50_{PKD1}$ (μM)
1	>300	>300
2	90	110
3	48	52
4	42	60
5	3.1	3.4
11β-dipropyl	2.6	2.9

The structure-activity study provided novel insights into the structural requirements for activity against PKD cells. Compounds **1** and **2**, which feature simpler linkers [carbamate-only (**1**), or secondary amine-only (**2**)], were significantly less active than compounds having both functional groups in the linker. This result suggested that the bifunctional linker is important for activity, with the amine functionality ostensibly helping with solubility and charge, and the carbamate providing conformational rigidity. Compounds **3** and **4** provided insight into the requirements for the left-hand side of the molecule. The aromatic ring alone (compound **3**) or the aliphatic dimethyl amine (**4**) were not as effective as the dimethyl-aniline compound **5**, which features both structural features. Moreover, compound **5** showed a very similar activity to that of 11β-dipropyl, suggesting that the length of the alkyl groups on the tertiary aniline is not a significant contributor to the toxicity of 11β-dipropyl.

One puzzling observation that emerged from the structure-activity study was that none of the compounds (including 11β-dipropyl) showed a consistent selectivity towards the $PKD1^{-/-}$ strain of the LLC-PK cells. The IC50 values are relatively close for the less active compounds (**2**,**3**,**4**) and statistically indistinguishable for the most active compounds (**5**, 11β-dipropyl). Moreover, the trend suggests the wild-type strain is more sensitive than the mutant. This is in contradiction with prior data that showed reproducible selectivity for both 11β-dichloro and 11β-dipropyl towards the mutant cell line. To address these conflicting observations, before we repeat the structure-activity study in the next few months, we will re-characterize the cell lines and evaluate whether a genetic drift or rescue mutation might have occurred in the mutant line.

d. Investigation of the mechanism of toxicity of 11β-analogs: ROS generation. (Essigmann, MIT)

Our previous work has shown that 11β compounds (specifically 11β-dichloro) kill cells by deregulating mitochondrial respiration, which leads to increased oxidative stress and by increasing the level of ER stress, which manifests as the unfolded protein response (UPR). Either of these perturbations may be influencing and promoting the other, with the end result being cell death by apoptosis.

A preliminary study was setup to determine the ability of the 11β-dipropyl analogs to induce ROS generation. Cells were exposed to compounds (**2-5**) at concentrations near their IC50 for 4 h. The compounds were washed off and then the cells treated with the ROS dye CM-H_2DCFDA, a compound that upon cellular uptake and oxidation becomes fluorescent. The median fluorescence for each treatment condition was then evaluated by flow cytometry and compared with the median fluorescence of vehicle (DMSO) treated cells. Preliminary results indicated that compounds **2, 5** and 11β-dipropyl at doses of 100 µM, 3 µM and 3 µM, respectively generated an increase in fluorescence (ROS), while compounds **3** and **4** at doses of 50 µM each had no effect. Further investigation, which includes testing of several doses of each compound, is underway to confirm these observations and quantify the amount of ROS induction.

e. Examining the mechanism of toxicity of 11β compounds in tissues. (Somlo, Yale).

The previous mechanistic study of 11β toxicity demonstrated the ability of the compound to localize to the mitochondrion, disrupt the flow of electrons through the ETC and induce formation of ROS (4). Therefore, we first investigated whether 11β induces ROS in cystic kidneys. Early Pkhd1-Cre cystic kidneys were harvested and stained for lipid peroxidation biomarker 4-hydroxynonenal (4-HNE), a common oxidative stress-induced cellular byproduct. Recent evidence suggests that the bulk of 4HNE in a cell is formed from the oxidation of mitochondria-specific phospholipid cardiolipin (5), and thus, it primarily reflects mitochondrial oxidative stress (6). First we established the specificity of the anti-4-HNE antibody by treating IMCD3 cells (an established kidney cell line) with antimycin A (a known ROS stressor) and 11β-dichloro (Figure 5); both drugs elicited a positive 4-HNE signal compared with DMSO treated cells.

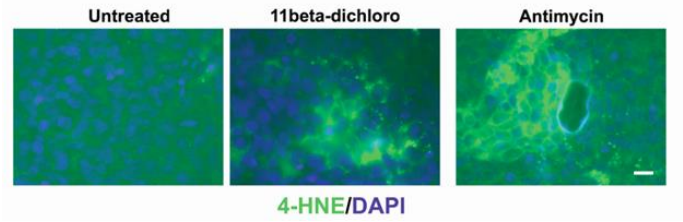

Figure 5. Detection of 4-HNE induced by 11β-dichloro treatment in cells. The green fluorescence denotes 4-HNE staining. The nuclei are stained with DAPI.

Next we investigated the status of 4-HNE in the 11β-treated mice. In the neonate model, the levels of 4-HNE were substantially increased in the cystic kidneys treated with 11β (Figure 6), but only in the DBA positive cells (i.e., $Pkd1^{-/-}$ cells), suggesting a specific induction of oxidative stress in the cystic cells. By contrast, no 4-HNE signal was detected in wild-type (proximal tubules) epithelia in the 11β-treated kidneys, or in any of the vehicle-treated kidneys. These observations are consistent with and bolster previous observations (described in the proposal for this project) that the transcriptional levels of oxidative stress inducible genes catalase (CAT) and Cu-Zn superoxide dismutase (SOD1) were significantly increased in the cystic kidneys treated with 11β.

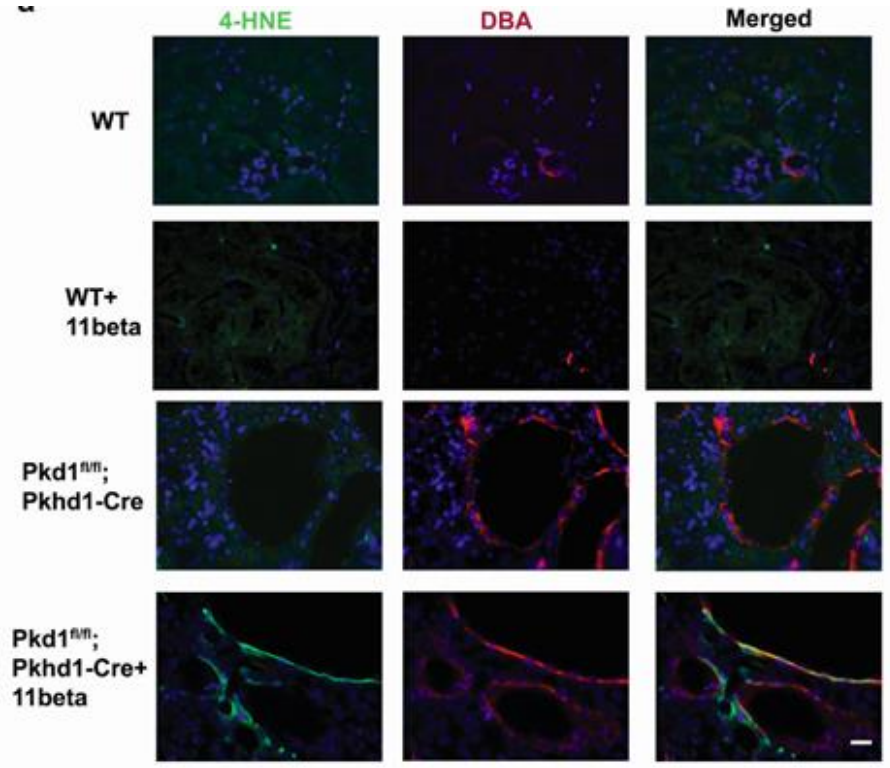

Figure 6. 11β-dichloro induces specific accumulation of the oxidative stress biomarker 4-HNE in cystic cells of the neonate mouse model of PKD ($Pkd1^{fl/fl}$; Pkhd1-Cre). DBA staining is characteristic for the collecting ducts that harbor the mutant cells.

The Pax8 adult model also recapitulated these mechanistic insights; cystic cells displayed higher level of 4-HNE staining (Figure 7), and catalase mRNA levels were significantly increased in treated whole kidney extracts (data not shown).

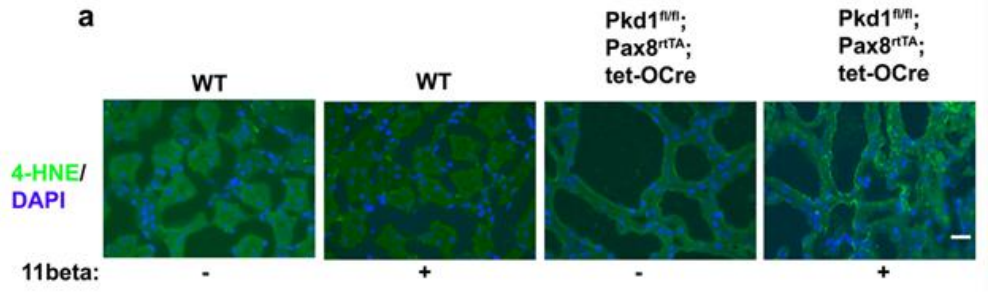

Figure 7. 11β-dichloro induces specific accumulation of the oxidative stress biomarker 4-HNE in cystic cells of the adult mouse model of PKD ($Pkd1^{fl/fl}$; $Pax8^{rtTA}$;tet-OCre). Cell nuclei are stained with DAPI.

f. Investigating the efficacy of 11β-dipropyl in the adult onset PKD mouse mode (Somlo, Yale).

Testing 11β-dipropyl activity in the adult onset PKD mouse model ($Pkd1^{fl/fl}$; $Pax8^{rtTA}$;tet-OCre) has been underway in the last funding period. The long duration of the assay (>24 weeks) and several setbacks related to animal husbandry have precluded analyzing a sufficient number of animals (vehicle vs 11β-dipropyl-treated) to formulate a statistically robust conclusion at this point. However, preliminary observations do suggest that 11β-dipropyl is effective in slowing down cystogenesis in this animal model, as indicated by decreased kidney/body weight ratio, decreased average cystic index and improved kidney function (i.e., lower levels of blood urea nitrogen) in the treated animals (Figure 8). Analyses of more animals in both groups (vehicle vs 11β-dipropyl treated) will continue in the current funding period to achieve the sample size and statistical power to draw robust conclusions.

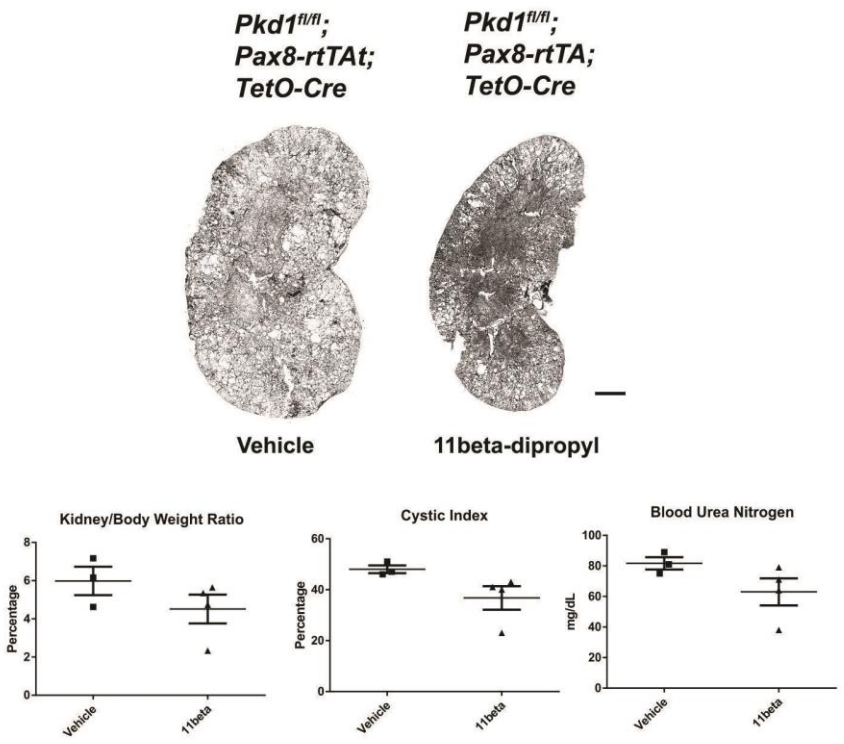

Figure 8. 11β-dipropyl ameliorates cystic disease in the adult mouse model of PKD ($Pkd1^{fl/fl}$; $Pax8^{rtTA}$;tet-OCre). The top part of the figure shows cross-sections of typical kidney specimens from vehicle and 11β-dipropyl treated animals. The bottom part shows the quantification of the current data (n=3 controls, n=4 treated) in terms of kidney/body weight ratio, cystic index and blood urea nitrogen. The dots denote individual animals; large bar indicates the mean, and error bars are +/- 1 SD. While the sample sizes are too small to formulate a statistical conclusion, the trends suggest 11β-dipropyl has a therapeutic effect in this mouse model.

What opportunities for training and professional development has the project provided?

A postdoctoral research scientist, Sakunchai Khumsubdee was supported by the project. A Northeastern Coop undergraduate student (Jake Campolo) and a master of science student from Université de Grenoble Alpes (Grenoble Alps University), France (Marie Gaillard) also contributed to the project. These students fully engaged in the scholarly enrichment activities of the MIT Departments of Chemistry and Biological Engineering and the Center for Environmental Health Sciences.

How were the results disseminated to communities of interest?

Results from this project were disseminated in the form of oral and poster presentations at the 2016 and 2017 (upcoming) annual meetings of the American Society of Nephrology. The titles of the published abstracts are included in Section 6.

What do you plan to do during the next reporting period to accomplish the goals?

During the next reporting period, we shall continue our work to investigate the mechanism of action of 11β compounds against PKD cells in cell culture, and their efficacy in animal models of PKD. First, we shall complete the synthesis of the last 11β-dipropyl analog proposed (compound **6**), and repeat the structure activity-study. Concomitantly, we shall thoroughly characterize the genotype of the LLC-PK cell lines available and establish if a genetic drift or rescue mutation has occurred in the PKD1 mutant cell line. The outcome of this investigation will dictate the next steps regarding this cell model, which may include restarting the culture from an original (validated) stock and/or clonogenic purification of the correct genotype. We shall also introduce a re-expression cell line, in which an exogenous PKD1 gene is expressed in the PKD knockout background; this cell line will control for any off-target genetic changes or genetic drift that may have occurred during the preparation and selection of the $PKD1^{-/-}$ cell line.

Second, we shall characterize of the ability of the 11β-dipropyl analogs to induce oxidative stress and mitochondrial dysfunction. The ROS induction experiments will follow the outline described above (part d). The effect on the mitochondrial electron transport chain will be evaluated in isolated mitochondria using an oxygen sensor (Essigmann, MIT). Preliminary work on this assay is already underway.

Third, we shall complete the testing of the efficacy of 11β-dipropyl in the adult *Pkd1* inactivation model (*$Pkd1^{flox/flox}$;Pax8-rtta;TetO-Cre;* 12 weeks old) mouse model (Somlo, Yale).

Fourth, we shall perform a more comprehensive investigation of the transcriptional effects of 11β compounds, focusing on the 11β-dipropyl compound, both in cell culture (Essigmann, MIT) and in the animal models (Somlo, Yale). Preparation work to assemble a collection of probes specific for oxidative stress genes and other PKD specific genes (as part of a Qiagen RT^2 Profiler PCR Array) is currently underway. This probe set will be used to record and compare

specific transcriptional responses between cell culture and animal models, in an effort to understand the molecular steps by which 11β compounds induce apoptosis in PKD cells.

4. IMPACT: *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

This project is likely to make an impact in the area of therapeutics targeted at polycystic kidney disease (PKD). The 11β compounds show great efficacy in preventing cystic growth in mouse models, suggesting that they can be developed into clinical candidates. Furthermore, the 11β compounds work by inducing apoptosis in cystic cells, a mechanism of action relatively unique in the field of PKD. The concept of using drug-induced ROS generation to treat this disease is novel and, aside from our own laboratories, it could inspire others to develop ROS-generating drug candidates.

What was the impact on other disciplines?

The 11β compounds that will be developed in this project for treating PKD are likely to have an impact for the treatment of other diseases, including cystic diseases in other organs (i.e., liver) or proliferative diseases (i.e., cancer). It is noteworthy that 11β-dichloro has already shown efficacy against a number of tumor types in animal xenografts.

What was the impact on technology transfer?

The new 11β compounds and derivatives synthesized in this project have the potential to qualify for composition of matter and use patents for treating PKD and related cystic diseases. In this regard, a recent communication from the USPTO conveyed optimism that intellectual property on the 11β-dipropyl compound, which has shown much evidence of efficacy, will be issued. Issuance of intellectual property will be a step toward licensing to a company that can efficiently bring a drug candidate to clinical trials.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS: *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

We synthesized an additional 11β-dipropyl analog (compound **5**), in which the dipropyl groups of 11β-dipropyl were replaced with methyl groups. Among the analogs synthesized, this compound represents one of the smallest changes in the structure of the molecule; not surprisingly, its properties were very similar to the properties of 11β-dipropyl, suggesting that the length of the alkyl chains attached to the aniline nitrogen contribute very little to the activity of the molecule.

Actual or anticipated problems or delays and actions or plans to resolve them

As outlined in part d, the LLC-PK cell lines used in the structure activity study gave inconsistent results, prompting the suspicion that either a genetic drift, rescue mutation or another genetic confounder has occurred during the experiment. Currently, assays involving the 11β-dipropyl analogs in this cell line model are on hold until the identity of the cell lines is confirmed. We plan to purify the mutant strain by dilution to single cells, and clonogenic growth, followed by a thorough characterization of the resulting clones. Additionally, we plan to generate a re-expression cell line, in which the PKD1 gene is re-introduced in the cell on a plasmid. If the re-expression cell line recapitulates faithfully the properties of the wild-type strain, it will engender further confidence in the identity and stability of the PKD1 mutant strain.

Changes that had a significant impact on expenditures

While the change outlined above involved the synthesis of a new (additional) compound, the impact on expenditures was negligible, because the starting materials were readily available.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Publications, conference papers, and presentations

Journal publications.

A manuscript that includes most of the preliminary data outlined in the proposal for this award, as well as some of the more recent data, is essentially complete and will be submitted within the next few weeks. The manuscript acknowledges the federal support received. The authors and title are as follows:

Fedeles BI, Fedeles SV, Ishikawa Y, Khumsubdee S, Campolo J, Rodrigues D, Gallagher AR, Westerling P, Krappitz M, Croy RG, Essigmann JM, Somlo S. “A synthetic anti-tumor agent ameliorates polycystic kidney disease by promoting apoptosis of cystic cells through increased oxidative stress” (in preparation).

Books or other non-periodical, one-time publications.
Nothing to report.

Other publications, conference papers, and presentations.

American Society of Nephrology published abstract:

1. Fedeles, S., Ishikawa Y., Gallagher R., Lee AH., Somlo S. Genetic interaction between *XBP1* and *Pkd1* modulates cyst progression in ADPKD. 2016, *J.Am.Soc.Nephrol.* (27); 211-12A

Website(s) or other Internet site(s)
Nothing to report

Technologies or techniques
Nothing to report

Inventions, patent applications, and/or licenses
The non-provisional patent application 14/515,441, entitled “Methods for treating polycystic kidney disease and polycystic liver disease” was filed jointly by MIT and Yale on October 15, 2014. While this application was filed prior to the start of the funding for this project, it covers a broad range of compounds that could be used to treat polycystic kidney disease, including the lead compound 11β-dipropyl. Very recent communication from the attorney team handling this application informed us that the USPTO patent examiner has agreed to allow a number of the claims put forth in the patent application and that the patent will be issued in the near future.

Other Products
Nothing to report.

7. Participants and Other Collaborating Organizations

Name:	*John M. Essigmann*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	*Ebrap ID* 237355
Nearest person month worked:	*1*
Contribution to Project:	*John Essigmann has the overall supervisory responsibility for the MIT site of this grant, including organizing deployment of personnel and the preparation of manuscripts and reports. Additionally, he helps interpret the data emerging from the project, especially data that involve the impact of the compounds made on oxidative stress and disruption of classical metabolic pathways.*
Funding Support:	

Name:	*Robert Croy*
Project Role:	*Research Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Dr. Croy provided supervision and guidance for the synthesis of 11β-dipropyl and analogs required for structure activity studies. He designed and performed quality control chromatographic analyses of the compounds. He also participated in conference calls to monitor experimental progress and coordinate with our partners at Yale.*

Funding Support:	

Name:	*Bogdan Fedeles*
Project Role:	*Research Scientist*
Researcher Identifier (e.g. ORCID ID):	ORCID: 0000-0001-5252-826X
Nearest person month worked:	*4*
Contribution to Project:	*Dr. Fedeles has designed, performed and coordinated the cell biology studies aimed at characterizing the efficacy and mechanism of 11β compounds in cell culture models. These included toxicity assays, ROS assays, isolated mitochondria assays, and transcriptional profiling using qPCR. He also performed quality control analysis for all the materials shared with the Yale collaborators.*
Funding Support:	

Name:	*Sakunchai Khumsubdee*
Project Role:	*Postdoctoral Affiliate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*7*
Contribution to Project:	*Dr. Khumsubdee has performed the organic syntheses of the 11β compounds, including the optimization of various synthetic steps, purification and physico-chemical characterization of the various intermediates and end products.*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Updated Other Support Pages are included in the Appendix.

What other organizations were involved as partners?

This is a COLLABORATIVE AWARD. Our collaboration partner is Stefan Somlo at Yale University. Details are below:

- **Organization Name:** Yale University
- **Location of Organization:** New Haven, CT, USA.
- **Partner's contribution to the projec**t
    - **Financial support:** none
    - **In-kind support:** development of cell lines for research
    - **Facilities**: none
    - **Collaboration:** Development of mouse models for PKD; designing and performing mouse model studies with the 11β compounds; mechanistic studies on mouse tissues and cells;
    - **Personnel exchanges**: none
    - **Other:** none.

9. SPECIAL REPORTING REQUIREMENTS

This is a COLLABORATIVE AWARD. An independent report from BOTH the initiating PI and Collaborating PI will be provided. The current report is from the Collaborating PI (John Essigmann, MIT). Given the collaborative nature of the work, experiments that involve materials and expertise provided by both institutions are included in this report. In each case, the responsible PI and the site where the work was performed is included.

10. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report.* ***DO NOT RENUMBER PAGES IN THE APPENDICES.***

Appendix 1: References

Appendix 2: John Essigmann Updated Active Support Pages

Appendix 3: Robert Croy Updated Active Support Pages

APPENDIX 1

REFERENCES CITED

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2. Horvath,C., Melander,W. and Molnar,I. (1977) Liquid chromatography of ionogenic substances with nonpolar stationary phases. *Anal. Chem.*, **49**, 142–154.

3. Hull,R.N., Cherry,W.R. and Weaver,G.W. (1976) The origin and characteristics of a pig kidney cell strain, LLC-PK. *In Vitro*, **12**, 670–677.

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5. Liu,W., Porter,N.A., Schneider,C., Brash,A.R. and Yin,H. (2011) Formation of 4-hydroxynonenal from cardiolipin oxidation: Intramolecular peroxyl radical addition and decomposition. *Free Radic. Biol. Med.*, **50**, 166–178.

6. Xiao,M., Zhong,H., Xia,L., Tao,Y. and Yin,H. (2017) Pathophysiology of mitochondrial lipid oxidation: Role of 4-hydroxynonenal (4-HNE) and other bioactive lipids in mitochondria. *Free Radic. Biol. Med.*, **111**, 316–327.

APPENDIX 2

ACTIVE SUPPORT

ESSIGMANN, John M.

ACTIVE SUPPORT

This project, previously reported as Active, has ended.

Title:	**Developing Solutions for Human and Environmental Health Risks Created by Exponentially Increasing Exposures to Lanthanide Metals**
Effort:	None reported
Supporting Agency:	MIT Environmental Solutions Initiative
Grants Officer:	Amanda Graham, agraham@mit.edu
Performance Period:	6/1/2015 - 8/31/2017
Funding Amount:	This is a three-lab collaborative program (with H. Hemond and B.
Project Goals:	Engelward) combining geochemistry and toxicology to assess potential risks to the environment and human health created by the lanthanide elements, which have become widely used in recent years and are essential to maintain technological innovation.
Specific Aims:	The Aims of this project are (1) to evaluate the levels of lanthanides in a local wetland and in air samples collected in 1995 and calculate deposition velocities (transfer functions) that estimate the levels of exposure of people to these agents and (2) to evaluate the toxicological effects (e.g., mutations and DNA strand breaks) in the genomes of cells exposed to realistic levels of these envrironmental toxicants.
Overlap:	No overlap.

This project, previously reported as Pending, has been funded.

Title:	**Science and Engineering for Sensors, Mechanisms, and Biomarkers of Exposures**
Effort:	2.30 calendar (Entire project)
Supporting Agency:	NIH/NIEHS
Grants Officer:	Bryann Benton, benton@mail.nih.gov
Performance Period:	8/1/2017 - 3/31/2022
Funding Amount:	This is a sub-project within a larger context of a Superfund
Project Goals:	Research Project proposal. The major goal of this sub-project is to reveal the mutagenic biomarkers that reflect risk factors of susceptibility to N-

	nitrosodimenthylamine (NDMA) and benzo(a)pyrene (BP), environmental contaminants found at Superfund sites.
Specific Aims:	The Aims of this sub-project are to determine if a special mouse model of cancer originally developed to study the mutagenic effects of aflatoxin B_1 can distinguish between the mutational spectra of two different environmental toxicants (NDMA and BP), alone or in combination.
Overlap:	No overlap.

ACTIVE

Title:	**The Environment as a Variable to Calibrate Mouse Models of Human Disease**
Effort:	0.50 summer
Supporting Agency:	NIH
Grants Officer:	Barbara Gittleman, gittlemanbj@niehs.nih.gov
Performance Period:	9/1/2013 - 7/31/2018 (no-cost ext.)
Funding Amount:	Study of life course variables as they reflect cancer susceptibility.
Project Goals:	This grant uses a unique animal model in which it is possible to use mutations as a biomarker to predict end stage tumors induced by aflatoxin B1. The grant focuses on the use of next-generation sequencing to probe the molecular steps in hepatocellular carcinogenesis. It also seeks to understand why males are more sensitive to females and young animals more sensitive than adults to this carcinogen. .
Specific Aims:	No overlap.
Overlap:	

Title:	**MIT Center for Environmental Health Sciences**
Effort:	2.40 calendar
Supporting Agency:	NIH/NIEHS
Grants Officer:	James William, williamsjr@niehs.nih.gov
Performance Period:	4/1/2016 - 3/31/2021
Funding Amount:	Core Center focused on the impact of the environment on human health and the health of the human ecosystem.
Project Goals:	This core grant provides support for the administrative structure, community engagement activities and core facilities for the Center for Environmental Health Sciences at MIT. Professor Essigmann is Center Director on this center core grant but does not receive any direct support. No overlap.
Specific Aims:	
Overlap:	

Title:	**Endogenous Nitrate Carcinogenesis in Man - Project 2**
Effort:	1.00 calendar
Supporting Agency:	NIH
Grants Officer:	Barbara A. Fisher, bfisher@mail.nih.gov
Performance Period:	6/1/2014 - 5/31/2019
Funding Amount:	Study of oxidative stress as it contributes of inflammation induced
Project Goals:	
cancer.	
Specific AIms:	The aim of this project is to identify a population of oxidative stress mediators that in aggregate represent the drivers of genetic changes many researchers believe underpin the conversion of normal cells to cancer cells. Specific attention is given to chemicals that cause oxidative stress associated with inflammation induced by nitric oxide, HOCl acid and related oxidants.
Overlap:	No overlap.

Title:	**The Therapeutic Effect of the Antitumor Drug 11beta and Related Molecules on Polycystic Kidney Disease**
Effort:	1.00 calendar
Supporting Agency:	U.S. Army Medical Research and Material Command
Grants Officer:	Elena G. Howell, elena.g.howell.civ@mail.mil
Performance Period:	9/30/2015 - 9/29/2018
Funding Amount:	To develop effective therapeutics of polycystic kidney disease
Project Goals:	Study of the mechanistic basis of activity of drug candidate molecules
Specific AIms:	that have selective activity against polycystic kidney disease in vitro and in vivo. Specific aims are to develop effective therapeutics of polycystic kidney disease.
	This grant.
Overlap:	

Title:	**Intra and Extra-Chromosomal Probes for Mutagenesis by Carcinogens**
Effort:	1.00 calendar
Supporting Agency:	NIH
Grants Officer:	Joy Kearse, kearsej@mail.nih.gov
Performance Period:	7/6/2016 - 6/30/2021
Funding Amount:	Study of mutagenic properties of DNA adducts produced by compounds
Project Goals:	that cause human cancer.

Specific AIms: The aim of this Project is to investigate the mechanisms by which simple environmental alkylating agents and the potent human liver carcinogen aflatoxin B1 induce mutations. This project involves synthesis of short oligonucleotides containing organic compound-DNA adducts. Typically the adducts are those of environmental agents such as vinyl chloride and short-chain alkylating agents. The oligonucleotides are inserted into the genomes of viruses or plasmids, which are replicated in cells. The type, amount and genetic requirements for mutagenesis of DNA damaging agent-derived adducts are characterized. .

Overlap: No overlap.

APPENDIX 3

ACTIVE SUPPORT

CROY, Robert C.

ACTIVE SUPPORT

This project, previously reported as active, has ended.

Title:	**Developing Solutions for Human and Environmental Health Risks Created by Exponentially Increasing Exposures to Lanthanide Metals**
Effort:	3.30 calendar
Supporting Agency:	MIT Environmental Solutions Initiative
Grants Officer:	Amanda Graham, agraham@mit.edu
Performance Period:	6/1/2015 - 5/31/2017
Funding Amount:	This is a three-lab collaborative program (with H. Hemond and B.
Project Goals:	Engelward) combining geochemistry and toxicology to assess potential risks to the environment and human health created by the lanthanide elements, which have become widely used in recent years and are essential to maintain technological innovation.
Specific Aims:	The Aims of this project are (1) to evaluate the levels of lanthanides in a local wetland and in air samples collected in 1995 and calculate deposition velocities (transfer functions) that estimate the levels of exposure of people to these agents and (2) to evaluate the toxicological effects (e.g., mutations and DNA strand breaks) in the genomes of cells exposed to realistic levels of these envrironmental toxicants.
Overlap:	No overlap.

This project, previously reported as pending, has been funded.

Title:	**Science and Engineering for Sensors, Mechanisms, and Biomarkers of Exposures**
Effort:	1.10 calendar
Supporting Agency:	NIH/NIEHS
Grants Officer:	Bryann Benton, benton@mail.nih.gov
Performance Period:	8/1/2017 - 3/31/2022
Funding Amount:	This is a sub-project within a larger context of a Superfund
Project Goals:	Research Project proposal. The major goal of this sub-project is to reveal the mutagenic biomarkers that reflect risk factors of susceptibility to N-nitrosodimenthylamine (NDMA) and benzo(a)pyrene (BP), environmental contaminants found at Superfund sites.

Specific Aims:	The Aims of this sub-project are to determine if a special mouse model of cancer originally developed to study the mutagenic effects of aflatoxin B_1 can distinguish between the mutational spectra of two different environmental toxicants (NDMA and BP), alone or in combination.
Overlap:	No overlap.

Title:	**MIT Center for Environmental Health Sciences**
Effort:	1.10 calendar
Supporting Agency:	NIH/NIEHS
Grants Officer:	James William, williamsjr@niehs.nih.gov
Performance Period:	4/1/2016 - 3/31/2021
Funding Amount:	Core Center focused on the impact of the environment on human health
Project Goals:	and the health of the human ecosystem.
Specific Aims:	This core grant provides support for the administrative structure, community engagement activities and core facilities for the Center for Environmental Health Sciences at MIT. Dr. Croy is co-director of the Genomics and Imaging Facilities Core. but does not receive any direct support.
Overlap:	No overlap.

Title:	**The Therapeutic Effect of the Antitumor Drug 11beta and Related Molecules on Polycystic Kidney Disease**
Effort:	8.80 calendar
Supporting Agency:	U.S. Army Medical Research and Material Command
Grants Officer:	Elena G. Howell, elena.g.howell.civ@mail.mil
Performance Period:	9/30/2015 - 9/29/2018
Funding Amount:	To develop effective therapeutics of polycystic kidney disease
Project Goals:	Study of the mechanistic basis of activity of drug candidate molecules
Specific AIms:	that have selective activity against polycystic kidney disease in vitro and in vivo. Specific aims are to develop effective therapeutics of polycystic kidney disease.
Overlap:	This grant.